Prognostic Value of Genes Related with Tumor Microenvironment in Ovarian Cancer

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Research article

Keywords: Ovarian cancer, tumor microenvironment, stromal scores, prognosis, TCGA

Posted Date: May 29th, 2020

DOI: <https://doi.org/10.21203/rs.3.rs-31574/v1>

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Abstract

Background Ovarian cancer (OV) is the fifth leading cause of cancer death among females. Growing evidence supports a key role of tumor microenvironment in growth, progress, and metastasis of OV. However, the impacts of gene expression signatures related with OV microenvironment on prognosis have not been well-established. This study aimed to apply ESTIMATE algorithm to extract genes related with tumor microenvironment that predicted poor outcomes in OV patients. **Methods** The gene expression profile of OV samples were downloaded from The Cancer Genome Atlas (TCGA) database. The immune scores and stromal scores of 469 OV samples were available based on the ESTIMATE algorithm. To better understand impacts of gene expression signatures related with OV microenvironment on prognosis, these samples were categorized based on their ESTIMATE scores into high and low score groups. A different OV cohort from the Gene Expression Omnibus (GEO) database was used for external validation. **Results** The molecular subtypes in OV patients were correlated with stromal scores, in which the mesenchymal subtype had the highest stromal scores ($p < 0.0001$). Poor prognosis were found in patients (especially for patients with overall survivals (OS) < 5 years) with higher stromal score ($p = 0.0376$). 449 differentially expressed genes (DEGs) in stromal scores group were identified and 26 DEGs were significantly associated with poor prognosis in OV patients ($p < 0.05$). Eventually, 6 genes have further validated to be significantly associated with poor outcomes in 40 patients from a different OV cohort of GEO database ($p < 0.05$). **Conclusion** In this study, several genes related with tumor microenvironment that predicted poor prognosis in OV patients were extracted. In addition, some previously overlooked genes could be potential prognostic biomarkers for OV.

Background

Ovarian cancer (OV) is a leading cause of cancer death among females [1]. It is characterized by a high degree of heterogeneity and a high risk of relapse, especially for high-grade serous ovarian carcinoma (HGS-OvCa) [2]. In 2018, there was about 22,240 new cases diagnosed and 14,070 deaths from the OV in the United States [3].

As the most lethal gynecological malignancy, OV tissue includes not only tumor cells but also non-tumor cells in the microenvironment. Tumor microenvironment represents a cellular environment where the tumor exists, consisting of immune cells, stromal cells, extracellular matrix component (EMC) and exosomes [4]. Among them, immune and stromal cells are major components. Growing evidence supports a key role of tumor microenvironment in growth, progress, and metastasis of OV, hence the prognosis [5]. However, the impacts of gene expression signatures related with OV microenvironment on prognosis remain unknown. Based on gene expression profiles, The Cancer Genome Atlas (TCGA) network classified OV into four molecular subtypes: differentiated, immunoreactive, mesenchymal and proliferative in TCGA database, which were associated with patients survival [6]. BRCA1 has an important role in DNA damage repair, chromatin remodeling, cell cycle control, and transcriptional regulation and BRCA2 mainly acts on homologous recombination[7]. Although there have been many reports about the

relationship between BRCA1/BRCA2 mutations and OV prognosis, the prognostic significance of BRCA1 mutations have not been well illuminated.

ESTIMATE (Estimation of STromal and Immune cells in MAlignant Tumor tissues using Expression data) has been proposed to be a new algorithm to score the fraction of immune and stromal cells in tumor tissues, then predict the infiltration level of immune and stromal cells and tumor purity based on gene expression signatures from the TCGA database [8]. Despite several reports have used the ESTIMATE algorithm in some tumors, such as breast cancer [9], head and neck squamous cell carcinoma [10], Glioblastoma multiforme [11], and gastric cancer [12], its application in OV has not been studied in detail.

In this study, we applied ESTIMATE algorithm to mine **genes** related with tumor microenvironment that predicted poor outcomes in OV patients. Notably, we have further validated these genes by a different OV cohort from the Gene Expression Omnibus (GEO) database (Series GSE32063).

Materials And Methods

Data Preparation

Level 3 data of gene expression profile of OV samples were downloaded from the TCGA data coordination center (<https://tcga-data.nci.nih.gov/tcga/>), using the Affymetrix HT Human Genome U133a (HT-HG-U133A) microarray platform on September 8, 2017. Clinical data of OV samples, including gender, age, histological type, clinical stage, neoplasm histologic grade, molecular subtypes, mutation status, and survival information were also obtained from TCGA database. ESTIMATE scores, including immune scores and stromal scores of 469 OV samples were available based on the ESTIMATE algorithm to the normalized expression matrix. A dataset from the GEO database were used for validation in this study, including 40 OV patients from Series GSE32063.

Identification and analysis of differentially expressed genes (DEGs)

According to the ESTIMATE scores obtained from the ESTIMATE algorithm, all OV samples were divided into two groups, namely high (immune or stromal) scores group and low (immune or stromal) scores group. R package limma was used for the identification of DEGs [13]. The DEGs between two groups (high group vs. low group) were screened based on the following cut-offs: false discovery rate (FDR) adjusted p-value < 0.05 and fold change (FC) > 1.5. “Upregulated DEGs” represented the genes that were upregulated in the high group compared with the low group and “downregulated DEGs” showed the genes that were downregulated in the high group compared with the low group.

Heatmaps and clustering analysis

ClustVis, as a web tool, was used to produce heatmaps and principal component analysis (PCA) plots of DEGs distribution between high and low ESTIMATE scores groups [14].

Construction of volcano plots

Volcano plots were used for determining the distribution of upregulated DEGs and downregulated DEGs.

Construction of Venn diagrams

The upregulated DEGs and downregulated DEGs shared in two groups (immune scores group vs. stromal scores group) were identified by Venn diagrams (Venn 2.1.0), respectively.

Gene ontology (GO) function and Kyoto Encyclopedia of Genes and Genomes (KEGG) pathway enrichment analysis of DEGs

GO function (biological processes (BP), molecular functions (MF), and cellular components (CC)) and KEGG pathway enrichment analysis of DEGs were assessed by the Database for Annotation, Visualization and Integrated Discovery (DAVID) [15]. An FDR adjusted p-value < 0.05 was considered to be statistically significant.

Construction of protein-protein interaction (PPI) network

The PPI network of upregulated DEGs in the stromal group was constructed by the STRING database [16] and Cytoscape software 3.6.1 [17].

Overall survival (OS) curve

The relationship between gene expression levels of DEGs and OS in OV patients was analyzed by Kaplan-Meier survival curve with a log-rank (Mantel-Cox) test.

Statistical Analyses

All statistical analysis were carried out using GraphPad Prism 5 (Version 5.01) and R version 3.5.1. The data analyses in this study were performed by standard statistical tests whenever appropriate. Comparisons between groups were evaluated by unpaired t test and one-way analysis of variance. $P < 0.05$ was deemed to be statistical significant.

Results

Correlation of ESTIMATE scores with OV prognosis

The gene expression profiles and clinical data of 469 OV female patients diagnosed pathologically between 1992 and 2009 were downloaded from the TCGA database. Among them, 63 (13.4%) cases were of differentiated subtype, 80 (17.1%) cases of immunoreactive subtype, 64 (13.6%) cases of mesenchymal subtype, 74 (15.8%) cases of proliferative subtype, and 188 (40.1%) cases of unknown molecular subtype. ESTIMATE algorithm was used to evaluate infiltration of immune and stromal cells and tumor purity. According to ESTIMATE algorithm, immune scores ranged from -1498.58 to 2774.16, and stromal scores were distributed between -1988.05 to 1837.43, respectively. In short, immune scores of patients were higher than stromal scores in the entire OV cohort. Immunoreactive subtype showed the

highest average immune scores of 4 subtypes, followed by mesenchymal subtype, differentiated subtype, and proliferative subtype (Fig. 1A, $p < 0.0001$). Similarly, mesenchymal subtype ranked the highest average stromal scores, followed by immunoreactive subtype, differentiated subtype, and proliferative subtype (Fig. 1B, $p < 0.0001$), suggesting that both immune and stromal scores were obviously correlated with the classification of molecular subtypes in OV patients. The proliferative subtype patients had the lowest immune scores and stromal scores.

In this study, there were 10 BRCA1 mutant patients with OV, 287 BRCA1 wild-type patients, and 172 patients of unknown status. In addition, there were 9 BRCA2 mutant patients, 288 BRCA2 wild-type patients, and 172 patients of unknown status. We drew the distribution of ESTIMATE scores between BRCA1/2 mutant and wild-type patients with OV. However, no significant difference was found in ESTIMATE scores between BRCA1/2 mutant and wild-type patients (Fig. 1C-F).

To better understand the correlation of ESTIMATE scores with OV prognosis, we categorized the 469 OV patients into high and low scores groups according to the top half of 235 samples with higher ESTIMATE scores and the bottom half of 234 samples with lower ESTIMATE scores. We found that patients with low immune scores showed no difference in median survival compared with patients with high scores ($p = 0.5707$, Fig. 1H). Median survival of patients with high stromal scores was lower than the patients with low scores based on Kaplan-Meier curve, although it showed no statistical significance ($p = 0.1145$). And the trend was mainly reflected in the shorter median survival (OS < 5 years) ($p = 0.0376$, Fig. 1G).

Comparison of DEGs with ESTIMATE scores

To explore the association of gene expression signatures with ESTIMATE scores, we compared gene expression profiles of 469 OV samples downloaded from TCGA database. Heatmaps and PCA plots demonstrated visualized distribution of gene expression profiles between high and low ESTIMATE scores groups in Fig. 2A-D. A total of 487 genes were identified to be DEGs in immune scores group. Among them, there were 442 upregulated DEGs and 45 downregulated DEGs in the high group compared with the low group of immune scores based on $p < 0.05$ and $FC > 1.5$. On the other hand, 449 genes were identified to be DEGs in stromal scores group. Of these, there were 428 upregulated DEGs and 21 downregulated DEGs in the high group compared with the low group of stromal scores based on $p < 0.05$ and $FC > 1.5$. Similarly, volcano plots (Fig. 2E, F) showed the distribution of upregulated DEGs and downregulated DEGs in immune scores and stromal scores groups. Furthermore, Venn diagrams displayed 287 upregulated DEGs shared in the immune scores and stromal scores groups, and 13 downregulated DEGs shared (Fig. 2G, H). Since only stromal scores showed distinct association with OV prognosis, we determined to mainly explore the DEGs in stromal scores group for the subsequent analysis in this study.

To predict the potential function of 428 upregulated DEGs (Table 1) in stromal scores group, we performed the GO function and KEGG pathway enrichment analysis of these genes. There were 355 GO terms of BP, 64 GO terms of CC, and 76 GO terms of MF indicated significant difference based on $FDR < 0.05$ and $-\log(FDR) > 1.301$. Top 10 GO terms were identified, such as immune response, extracellular space, and extracellular matrix (ECM) structural constituent (Fig. 3A-C). In addition, a total of 54 KEGG

pathway categories showed to be significant based on $FDR < 0.05$ and $-\log(FDR) > 1.301$. Top 10 KEGG pathway categories were identified, such as staphylococcus aureus infection, phagosome, ECM-receptor interaction, which were related with immune response (Fig. 3D).

Correlation of expression of DEGs with OV prognosis

To estimate the possible role of DEGs in OS, we plotted Kaplan-Meier survival curves with log-rank (Mantel-Cox) test. Among the 428 upregulated DEGs in the stromal scores group, a total of 44 DEGs were screen out to be significantly associated with OV prognosis. Of these 44 DEGs, 26 DEGs (hazard ratio (HR) > 1 , Table 2) obviously predicted poor outcomes for OV ($p < 0.05$, selected genes are shown in Fig. 4A-F).

PPI network of prognostic DEGs

To functionally explore the interactions between these prognostic DEGs, we constructed PPI network by the STRING database and Cytoscape software, which consisted of 41 nodes. We defined the color of node continuously by log (FC) value of prognostic DEGs in the network. Similarly, we identified the size of node continuously based on degree value which was the number of connections the node and other nodes. In this PPI network, MMP9, CXCL10, GZMB, CXCL9, CXCL11, CXCL13, C5AR1, GBP1, CD2, GBP2 and CX3CR1 were the notable nodes with higher degree values, because they showed strong association with other nodes (Fig. 5).

Validation of prognostic DEGs in a external cohort

To determine whether the prognostic DEGs identified from the TCGA database also have significant prognostic value in a different OV cohort, we obtained and analyzed gene expression signatures of a dataset with 40 OV samples from the GEO database (Series GSE32063). Eventually, 6 genes, including CH25H, CX3CR1, GFPT2, NBL1, TFPI2, and ZFP36 were verified to be significantly correlated with poor outcomes ($p < 0.05$, Fig. 6A-F).

Discussion

Because the early symptoms and signs of OV are atypical or non-existent, most patients are initially diagnosed at an advanced stage with low five-year survival rates [18]. Therefore, it is necessary to develop new methods to screen for early OV and new treatment strategies. With the impact of tumor microenvironment on OV prognosis has been increasingly emphasized [19], the interaction between tumor microenvironment components and OV prognosis was urgently needed to better understand. In this study, we identified several [genes](#) related with tumor microenvironment that predicted poor outcomes in OV patients from the TCGA database.

The mesenchymal subtype is featured by the overexpression of several genes related with activated stroma at the molecular level [20], although the stromal cells of the mesenchymal subtype have not been well described. It has been gradually recognized that stromal components are not only a scaffold for

integrity of tissue structure, but also play a role in tumor genesis, growth, invasion and metastasis [21,22]. In this study, the molecular subtypes in OV patients were found to be significantly correlated with the level of stromal scores, in which the mesenchymal subtype had the highest stromal scores. We further compared median survival of high and low stromal scores groups, and found that patients with high stromal scores were shown to be associated with low median survival, indicating poor prognosis. Therefore, our results demonstrated mesenchymal subtype was associated with poor outcomes, which was consistent with previous reports [23]. Stromal components in tumor microenvironment may significantly contribute to the behaviors of OV with mesenchymal subtype.

The prognostic significance of BRCA1/2 mutations in OV is still unclear. Previous report found a better prognosis for BRCA2 mutant patients, with no significant difference in prognosis for BRCA1 mutant patients compared with wild-type patients [24]. But several studies have showed that patients with BRCA1 and BRCA2 mutations had a better outcome compared with wild-type patients [25,26], whereas other studies have demonstrated no significant difference [27]. Our study also showed no significant prognostic difference, but small sample sizes of BRCA1/2 mutations might lead to inaccurate estimates of survival.

Increasing evidences have showed that tumor development is influenced not only by its internal tumor cells but also by its external tumor microenvironment components [28-31]. However, these studies have focused limited attention on stromal components in the tumor microenvironment. In this study, through gene expression profile of 469 OV samples, we firstly analyzed 449 DEGs identified from the comparison between high and low stromal scores groups, some of which participated in tumor microenvironment, as shown by GO function and KEGG pathway enrichment analysis. This was consistent with previous studies that stromal components were correlated with the building of tumor microenvironment in OV [32-34].

Then, we performed survival analysis of these 449 genes and determined that 44 DEGs were significantly associated with OV prognosis, of which 26 DEGs obviously predicted poor prognosis. In addition, we constructed PPI network of 44 DEGs and found that these genes were highly interrelated. [CX3CR1](#) [35], [CRYAB](#) [36,37], [TGFB1](#) [38], [TFPI2](#) [39], and [GFPT2](#) [40] have been reported to play a vital role in stimulating tumor proliferation, invasion, and metastasis in OV patients, predicting poor prognosis. Additionally, previous studies have shown that [PLK2](#) [41] and [MGP](#) [42] were associated with drug resistance.

Eventually, we have further validated 6 genes ([CH25H](#), [CX3CR1](#), [NBL1](#), [TFPI2](#), [GFPT2](#) and [ZFP36](#)) related with tumor microenvironment to be significantly associated with poor outcomes in 40 patients from a different OV cohort of GEO database. Of these 6 genes, as mentioned above, it has been demonstrated that the expression of [CX3CR1](#), [TFPI2](#) and [GFPT2](#) were associated with poor prognosis in OV patients. Although the remaining 3 genes, including [CH25H](#), [NBL1](#), and [ZFP36](#), have not previously showed correlation with OV prognosis, our results supported that these genes may be potential prognostic biomarkers for OV. As a reticulum-associated membrane protein, [CH25H](#) has been shown to inhibit

infection of several viruses through catalyzing cholesterol converting into 25-hydroxycholesterol (25HC) [43]. It has been previously demonstrated that the overexpression of NBL1 suppressed tumor growth [44]. Furthermore, ZFP36 has been reported to play a key role in the post-transcriptional regulation of tumor necrosis factor (TNF) and the modulation of mRNA stability [45].

Conclusion

we applied ESTIMATE algorithm based on stromal scores to extract several [genes](#) related with tumor microenvironment from TCGA database. Notably, we have further validated these genes by a different OV cohort from the GEO database, which may be significantly associated with poor prognosis in OV patients. In addition, some previously overlooked genes could be potential prognostic biomarkers for OV. However, there were also some limitations in this study. First, fewer genes have been shown to involve in OV prognosis, possibly due to the small sample size of OV cohort for validation. Second, considering that this was a retrospective study on the basis of public databases, it was difficult to include regional differences, since the database of this study was from the United States, while the database for validation was from Japan. Therefore, well-designed, prospective, and multicenter clinical studies are still imperative to further verify the prognostic value of these genes related with tumor microenvironment by large data-based analyses, which may help us to develop novel prognostic biomarkers and therapeutic targets for OV in clinical practice.

Abbreviation

OV: ovarian cancer; TCGA: Cancer Genome Atlas; GEO: Gene Expression Omnibus; OS: overall survivals; DEGs: differentially expressed genes; HGS-OvCa: high-grade serous ovarian carcinoma; EMC: extracellular matrix component; FDR: false discovery rate; PCA: principal component analysis; GO: Gene ontology; KEGG: Kyoto Encyclopedia of Genes and Genomes; BP: biological processes; MF: molecular functions; CC: cellular components; DAVID: Database for Annotation, Visualization and Integrated Discovery; PPI: protein-protein interaction; HR: hazard ratio; 25HC: 25-hydroxycholesterol; TNF: tumor necrosis factor.

Declarations

Acknowledgements

Not applicable.

Author contributions

Conception and design: Hui Zhi, Shimei Li, Shaoyun Hao

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All authors read and approved the final manuscript.

Funding

This work was supported by grant 201804010237 from the Science and Technology Program of Guangzhou, China.

Availability of data and materials

The datasets generated during the current study are available in the TCGA repository (<https://tcga-data.nci.nih.gov/tcga/>). TCGA are publicly available databases.

Ethics approval and consent to participate

All procedures performed in studies involving human participants were in accordance with the ethical standards of the institutional and/or national research committee and with the 1964 Helsinki declaration and its later amendments or comparable ethical standards. The study has been approved by the ethics committee of Sun Yat-Sen Memorial Hospital of Sun Yat-Sen University.

Consent for publication

Not applicable.

Competing interests

The authors declare no conflicts of interest.

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Tables

Table 1 428 Upregulated DEGs extracted from comparison between high and low stromal scores groups

Gene symbol	
1	THBS2
2	SERPINF1
3	VCAN
4	FN1
5	CTSK
6	DCN
7	COL5A2
8	FBN1
9	COL3A1
10	COL1A1
11	FAP
12	VCAM1
13	LUM
14	AEBP1
15	EVI2A
16	MMP2
17	COL5A1
18	RAB31
19	ACTA2
20	GLIPR1

21 COL6A3

22 COL10A1

23 POSTN

24 SPARC

25 TGFBI

26 CDH11

27 SNAI2

28 ECM1

29 COL1A2

30 PLA1

31 SFRP4

32 CRISPLD2

33 INHBA

34 OLFML2B

35 COPZ2

36 THBS1

37 LY96

38 COL11A1

39 FILIP1L

40 WIPF1

41 PMP22

42 COLEC12

43 CXCL12

44 CLEC2B

45 COL6A2

46 FCGR2A

47 PTPRC

48 DAB2

49 OLFML1

50 C3AR1

51 ITGA5

52 TNFAIP6

53 IL7R

54 CD163

55 GIMAP4

56 LRRC15

57 GMFG

58 EVI2B

59 HEPH

60 CD14

61 FMO1

62 LHFP

63 LGALS1

64 LAPTM5

65 GLT8D2

66 ECM2

67 CD53

68 GFPT2

69 VSIG4

70 EMP3

71 MS4A6A

72 FCER1G

73 SULF1

74 SAMSN1

75 PDGFRA

76 C1QB

77 ALDH1A3

78 AIF1

79 TAGLN

80 C1QA

81 F13A1

82 TDO2

83 SPHK1

84 TGFBI1

85 PDGFRB

86 PCOLCE

87 EBI2

88 ASPN

89 LPPR4

90 ARHGEF6

91 LCP2

92 ITGB2

93 MMP11

94 NBL1

95 CCL11

96 MS4A4A

97 EDNRA

98 CSF2RB

99 BGN

100 TMEM158

101 FCGR1A

102 GIMAP6

103 HCK

104 SLAMF8

105 C1S

106 SLA

107 IL10RA

108 PDLIM3

109 TYROBP

110 CSF1R

111 NNMT

112 GPNMB

113 ITGBL1

114 EPYC

115 PLEK

116 FYB

117 GREM1

118 SRGN

119 PSCDBP

120 CYBB

121 MNDA

122 CD93

123 TIMP3

124 FCGR2B

125 SPON2

126 COL16A1

127 ACTG2

128 SERPINE1

129 GLRX

130 HOPX

131 SRPX2

132 LY86

133 SLC31A2

134 ANGPTL2

135 LHFPL2

136 CCR1

137 OLFML3

138 PLXDC1

139 CXCL14

140 ARHGAP15

141 MYO1F

142 MAFB

143 C10orf56

144 LST1

145 KLF2

146 HNT

147 RCN3

148 PAPSS2

149 C5AR1

150 CD86

151 LOX

152 LOXL1

153 DOCK2

154 GUCY1A3

155 PLAUR

156 AQP1

157 GPR65

158 CXorf9

159 EPB41L3

160 NPL

161 NT5E

162 ARHGDIB

163 LYZ

164 TMEM176B

165 RGS1

166 NID2

167 RGS4

168 LILRB1

169 CD48

170 NUA1

171 FGL2

172 ISLR

173 PDPN

174 CCL4

175 IGSF6

176 SLC12A8

177 SH2B3

178 RNASE6

179 CST7

180 HMOX1

181 DACT1

182 HTRA1

183 CD2

184 DRAM

185 ELTD1

186 CCR5

187 TLR2

188 CLEC7A

189 SMPDL3A

190 CENTA2

191 PALLD

192 HCLS1

193 SCG2

194 DPYD

195 MPP1

196 TFEC

197 CD248

198 HEG1

199 FZD1

200 CNN2

201 IGFBP4

202 TLR7

203 ACSL1

204 CD4

205 DSE

206 NCF2

207 SRPX

208 IL2RB

209 GEM

210 CALD1

211 CD3D

212 CXCL9

213 ALOX5AP

214 COL4A1

215 RAC2

216 LXN

217 SLC7A7

218 TNFSF4

219 NID1

220 BASP1

221 IRF8

222 LPXN

223 C7orf10

224 RCAN2

225 CFD

226 IGKC

227 CCL5

228 LDB2

229 APOC1

230 CH25H

231 OMD

232 PTRF

233 AP1S2

234 SPARCL1

235 ADAMDEC1

236 IFI30

237 GZMA

238 MFSD1

239 GZMK

240 LTBP2

241 MGC14376

242 FOLR2

243 STAB1

244 P2RY5

245 RGS16

246 CCL2

247 MSN

248 F2R

249 DPYSL3

250 HLA-DPA1

251 RGS2

252 NKG7

253 CD36

254 PPAP2A

255 TMEM176A

256 COL15A1

257 C13orf15

258 IL2RG

259 MICAL2

260 CTGF

261 CD52

262 COMP

263 MMP9

264 RASSF2

265 HLA-DPB1

266 VIM

267 MOXD1

268 CTSS

269 BCL2A1

270 SEMA3C

271 CYR61

272 C1orf54

273 SPP1

274 MYLK

275 FAS

276 EFEMP1

277 SNX10

278 TREM2

279 APOC2

280 TPM1

281 FSTL1

282 CORO1A

283 PLVAP

284 GBP2

285 TNC

286 C1QTNF3

287 IL32

288 MARCKS

289 JAM3

290 ID3

291 ICAM1

292 TUBB6

293 TRIM22

294 CD69

295 HLA-DQB1

296 KDELR3

297 AOC3

298 CTA-246H3.1

299 TWIST1

300 IGLV2-14

301 TNFAIP3

302 FABP4

303 IGHM

304 CASP1

305 A2M

306 CILP

307 CTSO

308 ADH1B

309 MFAP5

310 GALNAC4S-6ST

311 LAMB1

312 CD44

313 SERPINA1

314 GJA1

315 EGR2

316 HLA-DRA

317 TPST2

318 MXRA8

319 CLEC5A

320 ITM2A

321 TMEPAI

322 PDGFD

323 KAL1

324 MXRA5

325 WIP1

326 BHLHB2

327 PLS3

328 COL4A2

329 PRKD1

330 IGKV1-5

331 CD74

332 GAS1

333 CYP1B1

334 CXCL13

335 FMO2

336 LCP1

337 IGLV3-25

338 DOCK4

339 SLC2A3

340 GZMB

341 GYPC

342 RARRES1

343 HLA-DRB1

344 C11orf75

345 SGK

346 ENPP1

347 CHN1

348 RFTN1

349 IL1B

350 HLA-B

351 MMD

352 HLA-DQA1

353 CFH

354 SLC46A3

355 MYL9

356 RARRES2

357 SOD2

358 IGF1

359 PYCARD

360 MAN2A1

361 ST3GAL1

362 GPX3

363 CXCL10

364 LIPA

365 KLF6

366 HLA-DMA

367 FKBP11

368 MGP

369 PDGFRL

370 PLK2

371 C3

372 GBP1

373 ZFP36

374 CCL18

375 PXDN

376 FER1L3

377 THY1

378 EMP1

379 CAV1

380 KRT6A

381 NOX4

382 KLK6

383 LTB

384 AMIGO2

385 IL15

386 APOL1

387 SQRDL

388 HLA-DMB

389 UBB

390 CXCL11

391 IER3

392 TMEM45A

393 SDC1

394 MMP12

395 C2

396 CRYAB

397 TPBG

398 CXCL1

399 AHNAK2

400 UBD

401 LRRC17

402 ADM

403 CX3CR1

404 FAT

405 PSMB9

406 S100A8

407 FOS

408 S100A9

409 HCP5

410 PTGDS

411 TFPI2

412 SLC34A2

413 QPCT

414 ST6GALNAC5

415 ANPEP

416 CLDN16

417 ZFPM2

418 MMP7

419 MMP1

420 MT1E

421 KLK7

422 DNAJC15

423 SPON1

424 INDO

425 KRT23

426 S100A2

427 PDZK1IP1

428 HP

Table 2 26 DEGs (hazard ratio (HR) > 1, p < 0.05) significantly predicting poor outcomes in OV

	Gene symbol	HR [exp(coef)]	P value
1	EMP1	1.191834	0.001404
2	CX3CR1	1.145661	0.001586
3	CH25H	1.268751	0.002297
4	VSIG4	1.141721	0.002867
5	PDGFRA	1.225756	0.004988
6	NBL1	1.154586	0.008032
7	TGFB1	1.135387	0.01367
8	C5AR1	1.203664	0.015185
9	HTRA1	1.134869	0.016343
10	NUAK1	1.118634	0.017457
11	FZD1	1.272641	0.01755
12	RGS4	1.220816	0.021435
13	ZFP36	1.118967	0.027078
14	OMD	1.20227	0.027353
15	PLK2	1.129302	0.028526
16	CLEC5A	1.154177	0.030876
17	ID3	1.134767	0.032007
18	CRYAB	1.084946	0.032917
19	TFPI2	1.074517	0.034037
20	CILP	1.090233	0.034052
21			

	MFAP5	1.075657	0.034413
22	COL16A1	1.143897	0.040232
23	PDPN	1.140548	0.042185
24	MGP	1.092676	0.044032
25	ALOX5AP	1.076064	0.044146
26	GFPT2	1.164861	0.045983

Figures

Figure 1

Correlation of ESTIMATE scores with OV prognosis. (A) Distribution of immune scores of OV subtypes (p < 0.0001). (B) Distribution of stromal scores of OV subtypes (p < 0.0001). (C) Comparisons of immune scores between BRCA1 mutant and wild-type patients (p = 0.0983). (D) Comparisons of stromal scores between BRCA1 mutant and wild-type patients (p = 0.3588). (E) Comparisons of immune scores between BRCA2 mutant and wild-type patients (p = 0.6634). (F) Comparisons of stromal scores between BRCA2

mutant and wild-type patients ($p = 0.3797$). (G) Kaplan-Meier survival curve for patients with low vs. high immune scores ($p = 0.5707$). (H) Kaplan-Meier survival curve for patients with low vs. high stromal scores ($p = 0.0376$).

Figure 2

Comparison of DEGs with ESTIMATE scores. (A) Heatmap of the distribution of gene expression profiles between high and low immune scores groups. Genes with higher and lower expression were shown in red

and blue, respectively, and genes at the same level of expression were showed in black. (B) Heatmap of the distribution of gene expression profiles between high and low stromal scores groups. (C) PCA plot of the distribution of gene expression profiles between high and low immune scores groups. (D) PCA plot of the distribution of gene expression profiles between high and low stromal scores groups. (E) Volcano plot of the distribution of upregulated DEGs and downregulated DEGs in immune scores group. Upregulated and downregulated DEGs were shown in red and green, respectively, the rest DEGs were shown in black. (F) Volcano plot of the distribution of upregulated DEGs and downregulated DEGs in stromal scores group. (G) Venn diagram showing the upregulated DEGs shared in the immune scores and stromal scores groups. (H) Venn diagram showing the downregulated DEGs shared in two groups.

Figure 3

GO function and KEGG pathway enrichment analysis of upregulated DEGs in stromal scores group. Top 10 GO terms of BP (A), CC (B), MF (C) and KEGG pathway categories (D) . P-values were adjusted by FDR.

Figure 4

Correlation of expression of DEGs with OV prognosis in TCGA. Kaplan-Meier survival curves of the selected DEGs were plotted by the comparison of groups between low (blue line) and high (red line) gene expression.

Figure 5

PPI networks of prognostic DEGs. The color of node was defined by log (FC) value of prognostic DEGs and the size of node was based on degree value.

Figure 6

Correlation of expression of DEGs with OV prognosis from the GEO database. Kaplan-Meier survival curves of the selected DEGs were plotted by the comparison of groups between low (blue line) and high (red line) gene expression.